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Ribosomal DNA variation in finger millet and wild species of *Eleusine* (Poaceae)

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Summary. Finger millet is an important cereal crop in the semi-arid regions of Africa and India. The crop belongs to the grass genus *Eleusine*, which includes nine annual and perennial species native to Africa except for the New World species *E. tristachya*. Ribosomal DNA (rDNA) variation in finger millet and related wild species was used to provide information on the origin of the genomes of this tetraploid crop and point out genetic relationships of the crop to other species in the genus. The restriction endonucleases used revealed a lack of variability in the rDNA spacer region in domesticated finger millet. All the rDNA variants of the crop were found in the proposed direct tetraploid ancestor, *E. coracana* subsp. *africana*. Wild and domesticated finger millet displayed the phenotypes found in diploid *E. indica*. Diploid *Eleusine tristachya* showed some similarity to the crop in some restriction sites. The remaining species were quite distinct in rDNA fragment patterns. The study supports the direct origin of finger millet from subspecies *africana*, shows *E. indica* to be one of the genome donors of the crop, and demonstrates that none of the other species examined could have donated the second genome of the crop. The rDNA data raise the possibility that wild and domesticated finger millet could have originated as infraspecific polyploid hybrids from different varieties of *E. indica*.

Key words: Finger millet – *Eleusine* – rDNA – RFLP – Grasses

Introduction

The genes coding for the 5.8S, 25S and 17S ribosomal RNA in plants are found in tandem repeats, varying in number from hundreds to several thousand copies (Rogers and Bendich 1987). Each repeat unit contains a large inter-genic spacer region (IGS) between the 17S and 25S genes. The IGS is comprised of subrepeats that vary in number and length, resulting in various IGS length units, referred to as variants. Size and restriction site variation in the rDNA spacer region has been examined in a number of plants including peas (Ellis et al. 1984), wheat (Dvorak and Appels 1986; May and Appels 1987), barley (Saghai-Maroof et al. 1984; Molnar et al. 1989), maize (Zimmer et al. 1988), rice (Cordesse et al. 1990; Sano and Sano 1990), broad beans (Rogers et al. 1986), and the sunflower genus (Choumane and Heizmann 1988). Variation in the spacer region has been useful for interpreting phylogenetic relationships among species. This study examines the IGS region variation in finger millet and related wild species.

Finger millet is one of the important cereal crops in the semi-arid regions of Africa and India. The crop belongs to the grass genus *Eleusine* Gaertn., which includes nine annual and perennial species that are native to Africa except for the New World species *E. tristachya* (Lam.) Lam. (Phillips 1972; Hilu and deWet 1976a). Finger millet is a tetraploid species with $2n=36$, $x=9$. Cytogenetic studies (Chennaveeraiah and Hiremath 1974; Hiremath and Chennaveeraiah 1982) suggest that finger millet is an allopolyploid, derived directly from the wild tetraploid *E. coracana* (L.) Gaertn. subsp. *africana* (Kennedy-O'Byrne) Hilu and deWet. Evidence from morphology, flavonoid chemistry and chloroplast DNA (cpDNA) nucleotide sequence similarities further substantiate the direct origin of the crop from subsp. *africana* (Hilu and deWet 1976a, b; Hilu et al. 1978; Hilu 1988). Our knowledge, however, regarding the origin of the genomes of the tetraploid species is incomplete.

Originally, the diploid species *Eleusine indica* (L.) Gaertn. was considered as the genomic donor of finger

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Table 1. Species and accessions of *Eleusine* used in the rDNA study. Sources of the plant material and the identification number (ID no) used in our seed collection are provided

Species	ID no	Sources of material
ssp. *coracana*	KH 2286	India, Tehri; U.S.D.A. P.I. 175139
ssp. *coracana*	KH 2291	India, Mandi; U.S.D.A. P.I. 271564
ssp. *coracana*	KH 2294	Uganda, Mugusu; U.S.D.A. P.I. 321126
ssp. *coracana*	KH 2295	Uganda, Fort Portal; U.S.D.A. P.I. 321130
ssp. *coracana*	KH 2296	Rhodesia, Salisbury; U.S.D.A. P.I. 225569
ssp. *coracana*	KH 2301	Ethiopia, Adiugn; U.S.D.A. P.I. 318897
ssp. *coracana*	KH 2317	India, Andhra Prades; Harlan & deWet #3810
ssp. *coracana*	KH 2322	India, Purulia; Harlan & deWet #3830
ssp. *coracana*	KH 2330	India, Bihar; Harlan & deWet #3860
ssp. *coracana*	KH 2347	India, Jammu; Harlan & deWet #3973
ssp. *coracana*	KH 2349	India, Kerala; Harlan & deWet #3980
ssp. *coracana*	KH 2355	India, Madras; Harlan & deWet #4007
ssp. *coracana*	KH 2366	India, Orissa; Harlan & deWet #4107
ssp. *coracana*	KH 2371	India, Rahasthan; Harlan & deWet #4154
ssp. *coracana*	KH 2381	India, Assma; Harlan & deWet #4231
ssp. *coracana*	KH 2385	India, Sikkim; Harlan & deWet #4264
ssp. *coracana*	KH 264	Kenya, Homa Bay; Hilu KH #264
ssp. *coracana*	KH 267	Tanzania, Altere; Hilu KH #267
ssp. *africana*	KH 212	Kenya, Nairobi; Hilu KH #212
ssp. *africana*	KH 225	Kenya, Handi Hills; Hilu
ssp. *africana*	KH 254	Kenya, Kisii; Hilu KH #254
ssp. *africana*	KH 268	Kenya, Mathana Valley; Hilu
ssp. *africana*	KH 2274	Nigeria, Zonkaw Farm; Harlan & deWet #1714
E. indica	KH 202	Kenya, Embu; Hilu KH #202
E. indica	KH 2303	India, Coonor; U.S.D.A. P.I. 217609
E. indica	KH 2304	S. Rhodesia, Salisbury; U.S.D.A. P.I. 226270
E. indica	KH 2315	Nigeria, Baissa; Harlan & deWet #3711
E. indica	KH 2393	Indonesia, Bogor; Harlan & deWet #5031
E. indica	KH 2452	China, Cauton; U.S.D.A. P.I. 408801
E. multiflora	KH 213	Kenya, Nairobi; Hilu KH #213
E. multiflora	KH 258	Kenya, Narok; Hilu KH #258
E. multiflora	KH 2422	Kenya, U.S.D.A. P.I. 226067
E. floccifolia	KH 242	Kenya, Kitale; Hilu KH #242
E. floccifolia	KH 2285	Ethiopia, S. of Addis, Ababa; U.S.D.A. P.I. 196853
E. floccifolia	KH 2395	Ethiopia, Alemeya; Harlan & deWet #5169
E. jaegeri	KH 203	Kenya, Aberdare Park; Hilu KH #203
E. jaegeri	KH 206	Kenya, Aberdare Park; Hilu KH #206
E. jaegeri	KH 207	Kenya, Aberdare Park; Hilu KH #207
E. jaegeri	KH 208	Kenya, Kinangop; Hilu KH #208
E. jaegeri	KH 217	Kenya, Molo; Hilu KH #217
E. jaegeri	KH 218	Kenya, Nakura; Hilu KH #218
E. jaegeri	KH 219	Kenya, Ojo Orok; Hilu KH #219
E. jaegeri	KH 220	Kenya, Nyahururu; Hilu KH #
E. jaegeri	KH 221	Kenya, Mau Narok; Hilu KH #221
E. jaegeri	KH 224	Kenya, Songor; Hilu KH #224
E. tristachya	KH 2283	Argentina, Santa Fe; U.S.D.A. P.I. 331791
E. tristachya	KH 2401	Argentina; deWet and Harlan #2315
E. tristachya	KH 2403	Brazil, Porto Allegre; deWet and Harlan #2566
E. tristachya	KH 2414	Uruguay; U.S.D.A. P.I. 477082
E. tristachya	KH 2464	Uruguay; U.S.D.A. P.I. 477078

millet (Greenway 1945; Kennedy-O'Byrne 1957; Jameson 1970). Based on a lack of chromosome pairing in a synthetic hybrid between *E. coracana* subsp. *coracana* and *E. indica*, Chennaveeraiah and Hiremath (1974) concluded that the latter species did not contribute any of the genomes of finger millet. On the other hand, a cpDNA sequence variation study of *E. indica* and the two subspecies of *E. coracana* suggested that *E. indica* could be one of the progenitors of finger millet (Hilu 1988). The chloroplast genome is maternally inherited in the majority of higher plants (Kirk and Tilney-Basset 1978; Sears 1983). Consequently, molecular evidence from the chloroplast genome can point out only the maternal parent. To determine the paternal progenitor and verify the possible allopolyploid origin of the crop, a bipaternally inherited marker from the nuclear genome, such as the rDNA spacer region, is needed. In this study, the size and restriction site variation in the nuclear rDNA region in finger millet and wild species of *Eleusine* were examined to provide information on the genomic origin of finger millet.

Materials and methods

Plant material

Seventy-three individual plants representing 50 accessions of domesticated and wild finger millet and five other species of *Eleusine* were used in this study (Table 1). Sample size for some

of the wild species was limited by the availability of seed collections. Two plants of each collection were grown in pots in the greenhouses. Leaf materials were collected from mature individual plants, frozen in liquid nitrogen, and stored at −70 °C. The seed materials are deposited in the seed collections of the senior author.

DNA isolation and analysis

Total cellular DNA was isolated from leaves following the procedure of Sano and Sano (1990). About 5 μg of DNA from each preparation was digested with the restriction endonucleases *Bam*HI, *Hin*dIII, and *Dra*I following the instructions of the supplier. The DNA fragments were separated electrophoretically on 0.8% agarose gels, stained in ethidium bromide, and photographed in UV light. Lambda *Hin*dIII and a one-kilobase (kb) fragment ladder markers (Bethesda Research Laboratories Inc, BRL) were included to estimate fragment sizes. The DNA fragments were transferred either to nitrocellulose membranes (Southern 1975) using the transfer kits supplied by BRL, or to Zetaprobe nylon membranes (BioRad Incorp) using the procedure of Reed and Mann (1985).

Two DNA clones covering the rRNA genes and their spacer regions were used as probes. The probes were kindly supplied by Yoshio Sano of the National Institute of Genetics, Mishima, Japan. The probes were *Bam*HI DNA fragments of rice (cv 'Taichung 65', Japonica type) cloned into pUC13. The pRY12 clone is 4.5 k in size and covers the spacer region between the 17S and 25S rRNA genes and the flanking regions (Sano and Sano 1990). Clone pRY18 is 3.8 kb in size and covers the remaining parts of the 17S and 25S rRNA genes, the 5.8S gene, and the small spacer regions between them. No cross hybridization was observed between pRY12 and pRY18 when they were used as controls in these experiments, indicating lack of sequence overlap and/or shared repetitiveness in the two probes. For the nitrocellulose membranes, the ECL Gene Detection System (Amersham Corp) was used for probe labeling and hybridization. To insure that the probe-bound horse-radish peroxidase was inactivated between probings, the membranes were washed and heated at 70 °C for 15 min in 2 × SSC (SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate, pH 7.0). Excess 2 × SSC was then removed from the membrane by blotting it on filter paper before placing it into the next hybridization buffer mixture. For the nylon membranes, the probes were labeled with ^{32}P using a nick translation procedure (BRL). The membranes were hybridized overnight at 65 °C in 3 × SSC, 20 m*M* phosphate buffer, pH 7.0, 7% SDS, 10 × Denhardt's solution, and 100 μg/ml salmon sperm DNA. Membranes were exposed to Kodak XAR-5 films. The probes were stripped off the membranes after each hybridization by washing the membranes 3 times, 20 min each, in 0.1 × SSC, 0.5% SDS at 95 °C.

Results

Species-specific IGS (intergenic spacer region) phenotypes were evident in all species except for *E. indica*, which was indistinguishable from *E. coracana*. The number of restriction sites and the type of IGS repeat unit varied between and sometimes within species. There was only one restriction site for *Hin*dIII in the rDNA region of the *Eleusine* species, while one or more sites existed for *Bam*HI and *Dra*I (Figs. 1 and 2). The presence of one site per repeat unit was useful in the identification of the

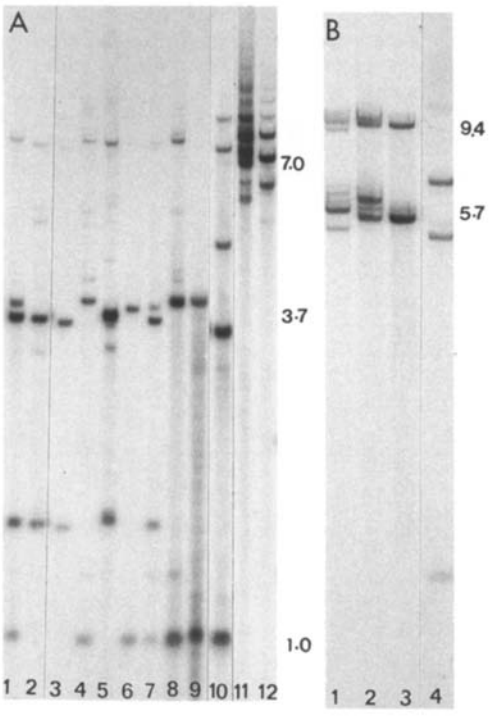

Fig. 1 A, B. Variation in *Bam*HI restriction site fragments of species of *Eleusine* detected by hybridization of the pRY12 rDNA probe from rice to total DNA digests. **A** *1–5 E. coracana* subsp. *africana*, *6–9 E. indica*, *10 E. tristachya*, *11–12 E. multiflora*. **B** *1–3 E. jaegeri*, *4 E. floccifolia*. The *top fragments* in **A** *1–10*, *13* and **B** *1–4* represent incompletely digested DNA

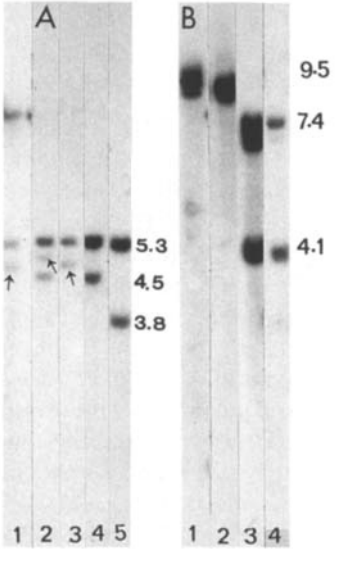

Fig. 2 A, B. Variation in *Dra*I restriction site fragments of species of *Eleusine* resolved by hybridization of the pRY12 rDNA probe from rice to total DNA digests. **A** *1–2 E. coracana* subsp. *africana*, *3–4 E. indica*, *5 E. tristachya*. **B** *1 E. multiflora*, *2 E. jaegeri*, *3–4 E. floccifolia*. *Arrows* point at DNA fragments that hybridized at lower intensities. Fig. 2A, lane 1 contains a high-molecular-weight band representing incompletely digested DNA

Table 2. *Bam*HI DNA fragments and phenotypes of the rRNA intergenic spacer region found in finger millet and wild species of the genus *Eleusine*. Fragment sizes are reported in kilobases. Letter of phenotypes refer to *E. coracana* subsp. *coracana* (C), *africana* (A), *E. indica* (I), *E. Tristachya* (T), *E. multiflora* (M), *E. jaegeri* (J), and *E. floccifolia* (F), while numbers accompanying the letters denote the different phenotypes found for each species

Phenotypes												
C1	A1	A2	A3	I1	T1	M1	M2	J1	J2	J3	F1	F2	F3
4.2	4.1	5.0	5.0	3.7	4.4	8.8	8.8	4.9	5.6	6.5	5.8	4.4	4.4
3.9	3.7	3.6	3.6	1.1	3.1	7.5	8.0		4.2	5.6	4.4	1.2	1.4
1.7	1.8	1.7	1.0		1.1	6.9	6.4		2.5	4.9	1.2		1.2
1.2	1.2					5.9	5.0						

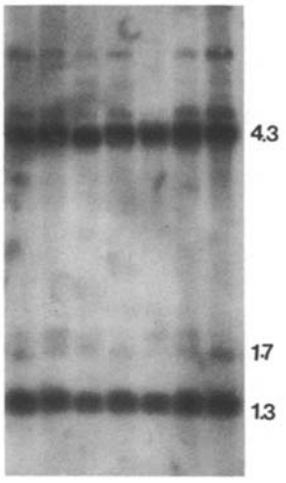

Fig. 3. *Bam*HI ribosomal DNA fragments similarities among African and Indian accessions of finger millet, *Eleusine coracana* subsp. *coracana*. The fragments were detected by using the pRY12 rDNA clone as a hybridization probe with total DNA digests

number of repeat length units and the estimation of their sizes.

Incomplete digestion of the DNA was observed in the experiments, which is probably due to different degrees of methylation. Methylation in the rDNA has been reported in a number of grasses such as wheat and barley (Gerlach and Bedbrook 1979; Appels et al. 1980), maize and its relatives (Zimmer et al. 1988), and rice (Cordesse et al. 1990).

Inconsistencies in repeat size produced by different enzymes were detected. Similar inconsistencies in size measurements have also been reported by Springer et al. (1989) and were attributed to the presence of secondary structures that might have affected the migration of the DNA bands.

Variation in BamHI restriction sites

*Bam*HI produced the largest number of rDNA fragments (Fig. 1, Table 2). Two to four DNA fragments were observed in the different species when the pRY12 clone was used as a hybridization probe (Table 2). The fragments ranged in size from 8.8 kb to 1.0 kb. The coding region probe (pRY18) cross-hybridized at different intensities with most of these DNA fragments and revealed an additional 4.7-kb fragment. The 4.7-kb, invariant fragment represents the coding region of the rDNA in the *Eleusine* species and is generated by two *Bam*HI restriction sites in that region. These *Bam*HI sites appear to be very conserved in the Poaceae since they are found across the family in species of wheat, rye, maize, tripsacum, sorghum, sugarcane, and rice (Gerlach and Bedbrook 1979; Apples et al. 1980; Zimmer et al. 1988; Springer et al. 1989; Sano and Sano 1990).

The *Bam*HI restriction fragment length polymorphism (RFLP) pattern revealed 14 phenotypes in the seven species of *Eleusine* (Table 2). The 19 accessions of finger millet from Africa and India were quite similar in RFLP patterns generated by *Bam*HI, differing only in the presence or absence of a very faintly hybridized fragment of about 1.77 kb (Fig. 3). The low intensity of hybridization of this fragment compared to the others implies that it is either found at low frequency at the individual plant level or has low sequence homology to the heterologous probe from rice. All DNA variants of domesticated finger millet (*E. coracana* subsp. *coracana*) were shared with those of *E. coracana* subsp. *africana* and *E. indica*. These latter two species shared the small fragment, approximately 1.1 kb, with *E. tristachya* (Fig. 1). The phenotype of the crop was quite similar to the A1 phenotype of wild finger millet. The finger millet phenotype and most of its variants are not found among the remaining species of *Eleusine* examined.

The annual African species *E. multiflora* A. Rich. differed from the remaining species of the genus by displaying rDNA fragments of 5.0–8.8 kb in a ladder fashion when pRY12 was used as a hybridization probe (Fig. 1, Table 2). The same DNA fragments hybridized at low intensities to the pRY18 probe; the invariant, 4.7-kb fragment was also detected by this probe. This ladder of fragments is characteristic of a partial digestion of tandemly repeated units. One to two major IGS length variants were found in the accessions of the perennial species *E. jaegeri* Pilg. and *E. floccifolia* (Forsk.) Spreng. (Fig. 1, Table 2). The rDNA fragments of the perennial species were different in size from those of wild and domesticated finger millet and the other annual species (Fig. 1).

Variation in DraI restriction sites

Four major phenotypes were revealed when the *Dra*I DNA digests were hybridized with the pRY12 probe

Table 3. *Dra*I and *Hin*dIII ribosomal DNA fragments found in species of *Eleusine* using the pRY12 and pRY18 ribosomal DNA fragments from rice as hybridization probes with total DNA digests

Species	*Dra*I													*Hin*dIII								
	9.4	9.0	8.8	8.6	8.4	7.4	7.1	5.3	5.0	4.7	4.5	4.1	3.8	10.0	9.8	9.6	9.5	9.3	7.9	7.2	6.8	6.4
coracana								+	+	+	+			+		+			+			
indica								+		+	+			+	+							
tristachya								+					+					+				
multiflora	+	+	+	+											+						+	+
jaegeri			+	+	+														+	+		
floccifolia						+	+					+	+			+	+					

(Fig. 2, Table 3). One phenotype showed two to three fragments per DNA digest and was characteristic of *E. coracana* – *E. indica* – *E. tristachya*. In this phenotype, a 5.3 kb fragment was common to all three species. The two subspecies of *Eleusine coracana* and *E. indica* possessed an additional 4.7-kb (Fig. 2a, lane 1 and 3) or 4.5-kb fragment (Fig. 2a, lane 2 and 4) representing two types of rDNA repeat length (Table 3). Accession KH274 of subspecies *africana* (Fig. 2a, lane 2) possessed a 5.0-kb variant in addition to the 4.5-kb fragment. These variants were not found in *E. tristachya*. Instead, *E. tristachya* displayed a 3.8-kb fragment. The two to three DNA bands generated by *Dra*I in *E. coracana*, *E. indica*, and *E. tristachya* are due to the presence of two restriction sites for that enzyme in each repeat unit (Table 2, Fig. 2a). This was confirmed by summing up the sizes of the bands and comparing them to the incompletely cut DNA that represents the whole repeat unit in their respective lanes. Further confirmation was also achieved by comparison with the sizes and the number of bands generated by *Hin*dIII (Table 3).

The second phenotype was characteristic of the annual species *E. multiflora* and is recognizable by its two or four DNA fragments ranging in size from 8.6–9.5 kb (Fig. 2b, lane 1). The third phenotype was found in the perennial *E. jaegeri*, where one or two 8.4–8.8 kb fragments that represent the whole repeat units were resolved in each individual plant (Fig. 2, lane 2). The fourth phenotype was characteristic of the perennial *E. floccifolia* with two large (7.1 kb and 7.4 kb) and two small (3.8 kb and 4.1 kb) fragments (Fig. 2b, lanes 3–4).

The coding region probe (pRY18 clone) cross-hybridized with all the variants of the four phenotypes resulting in the same RFLP patterns. However, the rDNA fragments cross-hybridized at different intensities to the two probes.

Variation in HindIII restriction sites

When the pRY12 probe was hybridized to the *Hin*dIII DNA digests, one to two variants in each sample were resolved (Table 3). The variants were large in size and all cross-hybridized to the pRY18 probe. The size and the cross-hybridization indicate that the variants represent the complete repeat units of the rDNA genes. The two variants observed in the *Dra*I DNA digests of accession KH274 of subspecies *africana* were confirmed by the *Hin*dIII RFLP pattern. The variants in *E. tristachya* were smaller than those of the *E. coracana-E. indica* group, corresponding to the differences in fragment sizes generated by the endonuclease DraI (Fig. 2a, lanes 1–4, Table 3). *Eleusine multiflora* displayed variants of different sizes (6.4 kb, 6.8 kb, and 9.8 kb) in different accessions (Table 3). The ladder type of fragment pattern was not apparent in this restriction enzyme digest of *E. multiflora*.

Discussion

One of the striking results in this study was the lack of variability in the rDNA spacer region in domesticated finger millet (Fig. 3). Only one basic phenotype was observed in all the collections examined. Variation was in a 1.77-kb fragment that appeared at a very low concentration in the probe hybridization experiment. This fragment could be a DNA sequence repeat in an IGS length variant present at low frequency in the plant tissues since variation in presence/absence and intensity of the DNA fragments existed among the accessions of the crop.

Finger millet was represented by accessions that cover its full geographic distribution in Africa and India. The results are particularly intriguing in light of the geographic and historic isolation between the African and Indian cultivars. Finger millet was domesticated in the highlands of East Africa around the third millennium B.C. or earlier (Hilu et al. 1979), which makes it one of the oldest domesticated plants in Africa south of the Sahara. India is a secondary center of diversity (Hilu and deWet 1976b). The crop is believed to have been introduced to southern India probably as early as the second millenium B.C. as archaeological records indicate (Hilu et al. 1979). From there, finger millet spread in all direc-

tions in India, resulting in the differentiation of new races (Hilu and deWet 1976b). Complete homogeneity among the races in the IGS region has prevailed in spite of the antiquity of the crop, its wide distribution over diverse geographic areas in two continents, the relatively early isolation of the Indian cultivars, and the strong artificial as well as natural selection imposed on the crop.

The lack of variability in the IGS region of the crop is in marked contrast to that of its wild progenitor (subsp. *africana*), where three phenotypes and nine variants were found in the five accessions examined. When the patterns of the IGS region in wild and domesticated finger millet are contrasted, it sems evident that the crop was domesticated from a very limited number of populations of the wild species. This finding underscores the very narrow genetic base of finger millet. Natural hybridization between finger millet and subspecies *africana* has been reported (Mehra 1962). This natural gene flow should have resulted in some degree of genetic diversity detectable at the IGS region. The lack of IGS phenotype diversity in the crop is possible if one assumes that the crop has been hybridizing consistently and selectively with wild species plants of the same IGS phenotype. However, wide distribution of the crop and the wild species, the extensive overlap of their geographic distribution, and the relatively high diversity in IGS phenotypes in the wild species do not support this assumption.

Lack of diversity in the crop can be explained by its domestication from a subset of the wild species and by the subsequent limited introgression between wild and domesticated finger millet because they are highly self pollinated. It is also possible that the IGS region in finger millet is under particular evolutionary constraints that prevented structural changes in its variants. The region could also have undergone extensive interlocus homogenization of its rRNA genes (Dvorak 1990). Variability in the IGS has been observed in various crops such as wheat, barley, rice, broad beans, peas, and soybean (Appels and Dvorak 1982; May and Appels 1987; Saghai-Maroof et al. 1984; Sano and Sano 1990; Rogers et al. 1986; Ellis et al. 1984; Choumane and Heizmann 1988).

The tetraploid wild finger millet and the diploid *E. indica* diplayed high variability in IGS phenotypes and variants (Fig. 2, Table 2). The two share all the *Bam*HI phenotypes observed in this study except the *E. indica* I_1 phenotype found in one accession from India. All the variants of *E. indica* generated by the *Dra*I endonuclease were found in *E. coracana* (Fig. 2a, Table 3). These results underscore the high phylogenetic affinities between the two species and further support the cpDNA finding (Hilu 1988) that singled out *E. indica* as a genome donor to tetraploid finger millet. Data from both cpDNA and rDNA are in disagreement with the cytogenetical study of Chennaveeraiah and Hiremath (1974) in which it was concluded that *E. indica* did not contribute any of the genomes of finger millet.

Chennaveeraiah and Hiremath (1974) expressed caution in their conclusion since their study was based on 45 pollen mother cells obtained from a single cross. S. C. Hiremath (personal communication, 1989) indicated that in three new hybrids produced between *E. indica* and *E. coracana* he observed nine bivalents and nine univalents in a large number of cells. This new cytogenetic finding is in line with the molecular markers data concerning the origin of one of the finger millet genomes from *E. indica*.

Since wild and domesticated finger millet (*E. coracana*) are considered allopolyploids (Chennaveerdiah and Hiremath 1974; Hiremath and Chennaveeraiah 1982), then the paternal progenitor of finger millet should be sought among the remaining diploid species. Among the other wild species included in this study, only *E. tristachya* appears to share some rDNA fragments with wild and domesticated finger millet (Tables 2, 3). The remaining species of the genus, including the diploid annual *E. multiflora*, look quite divergent. *Eleusine tristachya*, like the crop, is an annual with a basic chromosome number of 2 n = 9. Although the general restriction patterns generated by *Bam*HI in *E. tristachya* and *E. coracana* are similar, the two species shared only one fragment (approximately 1.1 kb) unique to this group of species. The two species also shared the 5.3-kb invariant *Dra*I fragment.

Therefore, the rDNA results point to some genetic affinities between these two species. However, there are a number of variants and restriction sites unique to *E. tristachya*.

If *E. tristachya* did contribute one of the two genomes of finger millet, then the variants and restriction sites unique to this genome should have been detected in the tetraploid crop and its wild progenitor subsp. *africana*. Evidence for Mendelian inheritance of the rDNA phenotypes has been cited in plants. Saghai-Maroof et al. (1984) have shown that variability in the rDNA in barley is governed by two independent, codominant alleles that were inherited in a simple Mendelian fashion. All the bands of the parental phenotypes in that study were recovered in the plants of the F_1 and F_2 generations. Similar results were also obtained in studies of the inheritance of the rDNA phenotypes in wheat and maize-teosinte (May and Appels 1987; Zimmer et al. 1988). Therefore, the distinctness between the phenotypes of the two species is not supportive of *E. tristachya* as one of the genomic donors of finger millet. The synthesis of diploid and allopolyploid hybrids between *E. tristachya* and *E. indica* and the study in these hybrids of chromosome pairing, mode of inheritance of the rDNA, and the behavior of the *E. tristachya* IGS region could provide more insight into the evolution of this crop. This is par-

ticularly important since Zimmer et al. (1988) also reported the non-additive inheritance of the rDNA spacer region in hybrids between *Zea* species.

Eleusine coracana and *E. tristachya* have different geographic distributions. Whereas wild finger millet, subspecies *africana*, is found in the wild only in East Africa, *E. tristachya* is native to the New World and found only as a rare adventive in limited locations in north eastern Africa (Phillips 1972; Clayton et al. 1974). The geography of distribution of the species of *Eleusine* suggests that the genus evolved and differentiated in Africa. Thus, the similarities between *E. tristachya* and finger millet in the rDNA could be a reflection of shared ancestry between *E. coracana*, *E. indica* and *E. tristachya* from the early stages of the differentiation of the genus. Results from an allozyme study of these species (Werth and Hilu, unpublished data) are in agreement with the cpDNA and rDNA data in showing *E. indica* but not *E. tristachya* as a genome donor to finger millet.

It is possible that wild finger millet could have originated from a cross between two genotypes of *E. indica*. Synthesizing infraspecific allopolyploid hybrids from different genotypes of *E. indica* and using them in crosses with wild and domesticated finger millet could provide valuable information on the origin of the two genomes of the crop.

Two other East African species of *Eleusine*, *E. intermedia* and *E. kigeziensis*, have not been included in this study because of the unavailability of seed collections. Both species differ from the crop and its wild progenitor in being perennials. However, their perenniality should not preclude them from being possible progenitors of the crop. A similar study of these two species could provide additional useful information on the origin of finger millet.

The rDNA study also revealed the general pattern of evolution in the genus *Eleusine*. Besides showing the similarities between *E. coracana*, *E. indica* and *E. tristachya*, it underscored the distinctness of the perennial species and the isolated position of *E. multiflora* in the genus. The position of *E. multiflora* in the genus was questioned by Phillips (1972) on the basis of morphology and by Hilu et al. (1978) using flavonoid distribution. *Eleusine multiflora* differs from the other species of the genus in the alternate rather than the digitate arrangement of spikes on the inflorescence axis. In addition, Phillips (1972) cited differences in mode of floret articulation, shape of lemma tip, and pattern of rupture of the membranous pericarp of the seed. She suggested *E. multiflora* as a link between *Eleusine* and another grass genus *Acrachne*. Based on morphology, flavonoid distribution, and rDNA data, we consider the placement of *E. multiflora* in the genus *Eleusine* to be questionable. The species, however, should be retained in *Eleusine* until its affinity to *Acrachne* is well understood.

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